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TITLE: Validation and Interrogation of Differentially Expressed and Alternatively Spliced Genes in African-American Prostate Cancer

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**6. AUTHOR(S)**

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**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT** These studies address the urgent need to elucidate the molecular mechanisms underlying the more aggressive prostate cancer biology in African American (AA) men. Specifically, our objectives are to 1) expand our sample cohort and delineate the relationship between genetic/epigenetic/post-transcriptional factors in AA prostate cancer and Gleason grade and 2) manipulate splicing using novel splice-switching oligonucleotides (SSOs) and determine functional outcomes. Toward these objectives, we have opened our GENomics of Cancer DisparitiEs Study to obtain AA and white prostate cancer blood and tissue specimens. For all tissue specimens collected, we have screened for tumor content, determined Gleason grade, isolated DNA and RNA and annotated. In addition, we have designed and synthesized SSOs to manipulate *PIK3CD* alternative splicing, with SSOs targeting the exon 23 junction or a putative enhancer to produce the AA *PIK3CD* variant and targeting a putative repressor to produce the white *PIK3CD* variant.

**15. SUBJECT TERMS**
Prostate cancer, health disparities among racial groups, molecular mechanisms, differential gene expression, alternative RNA splicing, epigenetic alterations, clinical tumor aggressiveness

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**INTRODUCTION**: African American (AA) men exhibit 2-fold higher incidence and 3-fold higher mortality rates from prostate cancer compared to white men. Although much of this disparity remains after controlling for factors related to access to care, very few studies have utilized this population-based difference to identify molecular mechanisms of tumor aggressiveness. The studies proposed here address the urgent need to elucidate the molecular mechanisms underlying the more aggressive prostate cancer biology in AA men. Our objectives are to 1) expand our sample cohort and delineate the relationship between genetic/epigenetic/post-transcriptional factors in AA prostate cancer and Gleason grade and 2) manipulate splicing using novel splice-switching oligonucleotides and determine functional outcomes. Establishing the underlying genetic/ epigenetic/post-transcriptional differences between AA and white prostate cancer and the biologic relevance of these differences to tumor biology will identify novel precision biomarkers and/or molecular targets for precision medicine interventions that will have profound implications for the prevention, screening, diagnosis and management of prostate cancer in AA men as well as men of all races with aggressive disease. Specifically, if positive, these genetic/epigenetic/post-transcriptional differences could be developed as prognostic markers, in the context of Gleason grade and other prognostic variables, to delineate patients at greater risk of progressing on active surveillance or through localized therapy. In addition, the causal relationship of these pathways would help to rationalize specifically targeted therapy in selected patients.

**KEYWORDS (20 words):** Prostate cancer, health disparities among racial groups, molecular mechanisms, differential gene expression, alternative RNA splicing, epigenetic alterations, clinical tumor aggressiveness

**ACCOMPLISHMENTS:**

**What were the major goals of the project?**

Task 1. Validate differentially expressed and spliced candidate genes in AA prostate cancer in an expanded sample cohort and define the relationship between these genes and Gleason grade. Months 1-21. 60% complete (please see progress for Task 1).

Task 2. Define the biologic significance of differences in *cis*-acting splicing elements of alternatively spliced candidate genes in AA prostate cancer to alternative splicing events specific to AA prostate cancer and define the relationship between these patterns and Gleason grade. Months 21-36. 25% complete (please see data for Task 2).

Task 3. Use splice-switching oligonucleotides to delineate the biologic relevance of race-related differentially spliced genes involved in PIK3CD and MET signaling. Months 1-24. 50% complete (please see data for Task 3).

**What was accomplished under these goals?**

**Progress for Task 1:** We continue to obtain individual patient AA and white prostate cancer tissue specimens and patient-matched blood specimens under our IRB approved GENomics of CAncer DisparitiEs (GENCADE) protocol and capture annotated data in our accompanying database. For all tissue specimens collected to date, we have screened the specimens for tumor content, determined the Gleason grade, isolated cellular DNA and RNA and confirmed adequacy of yield and quality of these nucleic acids for downstream analyses and annotated the specimens. For white prostate cancer specimens, we have completed our collection of Gleason 7 specimens and need to collect only two additional Gleason 6 specimens and only one additional Gleason 8-10 specimen to fully complete collection of all groups for downstream analyses. For AA prostate cancer specimens, six additional Gleason 6 specimens, five additional Gleason 7 specimens and eight additional Gleason 8-10 specimens remain to be collected to fully complete collection of all groups for downstream analyses. Our institution recently hired a new Department of Pathology chair, Dr. Jiaoti Huang. He had developed a new whole mount strategy to procure tissue from prostatectomies for research at his prior institution and he implemented this strategy at our institution the beginning of May of this year. Since implementation of this strategy, all of the specimens we have obtained have been of sufficient size and tumor content, which has dramatically accelerated our rate of collection and which will continue as we proceed with

collection of our remaining specimens. In addition, we have obtained IRB approval to collect specimens at our qualified collaborator's site, The George Washington University. This will enable us to accelerate our rate of collection even further.

**Data for Task 2:** While we continue to obtain individual patient AA and white prostate cancer tissue specimens and patient-matched blood specimens, we have completed two additional analyses regarding the clinical significance of differences in *cis*-acting splicing elements of alternatively spliced candidate genes in AA prostate cancer. To do this, we have used publically available data from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), the Multiethnic Cohort Study (MEC), the Breast and Prostate Cancer Cohort Consortium (BPC3) and the Ghana Prostate Study. These analyses have led to two submitted manuscripts. The first manuscript, "Variants of stemness-related genes predicted to regulate RNA splicing associated with racial disparities in susceptibility to prostate cancer", submitted to Carcinogenesis, reports our identification of 32 single nucleotide polymorphisms (SNPs) in five genes that are significantly associated with prostate cancer risk, of which six SNPs in three genes (*TP63*, *ALDH1A1* and *WNT1*) and eight *EGFR* SNPs showed heterogeneity between the two populations. Moreover, we report that our bioinformatics analysis has revealed that *EGFR* rs2072454 and linkage disequilibrium SNPs of the identified SNPs in *MET* and *ALDH1A1* ($r^2 > 0.6$) are predicted to play a role in RNA splicing regulation. Furthermore, we report the rs116458171A allele correlated with lower *MET* mRNA expression in Africans and the rs2072454C allele correlated with higher *EGFR* mRNA expression in Europeans. These variants may serve as novel biomarkers for disparity in prostate cancer risk. Please see ***Figure 1-4*** and ***Table 1-3*** at the end of this progress report.

The second manuscript, "Single nucleotide polymorphisms of stemness pathway genes predicted to regulate RNA splicing, microRNA and transcription are associated with prostate cancer survival", submitted to Clinical Cancer Research, reports our identification of SNPs of the *CD44* (rs9666607), *ABCC1* (rs35605 and rs212091), *GDF15* (rs1058587) and *ITGB1* (rs11009151) genes that are associated with prostate cancer survival and that are predicted to be functional. Specifically, we report a predicted role for rs9666607 of *CD44* and rs35605 of *ABCC1* in splicing regulation, rs212091 of *ABCC1* in miRNA binding site activity, rs1058587 of *GDF15* in causing an amino acid change in the GDF15 protein and rs11009151 of *ITGB1* in affecting transcription factor binding. These variants represent novel prognostic markers for overall survival of prostate cancer and support a contribution of the stemness pathway to prostate cancer patient outcome. Please see ***Figure 5-7*** and ***Table 4-6*** at the end of this progress report.

**Data for Task 3:** We have further assessed our novel chemically modified splice switching oligonucleotide (SSO) to correct aberrant splicing leading to production of AR-V7, an androgen receptor variant that lacks the ligand binding domain, is constitutively active and associates with castrate resistant prostate cancer, poorer clinical outcomes and resistance to androgen ablation/androgen receptor inhibition therapies (AR-V7 SSO). Preliminary data indicates transfection of a panel of white and AA prostate cancer cell lines with AR-V7 SSO decreases AR-V7 RNA and protein in a dose-dependent manner. In addition, preliminary data indicates this biochemical response correlates with biologically significant phenotypes, as AR-V7 SSO also decreases colony forming ability of prostate cancer cells derived from an AA patient. It is important to note that in this experiment a transfection efficiency of only 40-55% was achieved. Thus, it is likely that repetition of this experiment under optimized transfection conditions, recently achieved, will result in even more profound inhibition of colony forming ability by AR-V7 SSO. Moreover, preliminary data indicates the biochemical response also correlates with the biologically significant phenotype of proliferation, as AR-V7 SSO also inhibits proliferation of prostate cancer cells derived from a white patient. Furthermore, AR-V7 SSO also inhibits proliferation of these prostate cancer cells in the presence of enzalutamide, an androgen receptor inhibition therapy. Please see ***Figure 8*** at the end of this progress report.

Along with our qualified collaborator and his laboratory, we are preparing a manuscript focusing on our identification of a large number of alternative RNA splicing events in cancer-associated pathways in white versus AA prostate cancer, with a subset of these events also being detected in patient-matched normal prostate specimens. The events have biological significance, with one isoform of *PIK3CD* versus the alternative

associating with increased oncogenic signaling, proliferative and invasive phenotypes. In addition, the events have clinical significance as biomarkers and molecular targets for therapeutics, with one *PIK3CD* isoform versus the alternative associating with sensitivity or resistance to small molecule inhibition of the protein encoded by the alternatively spliced gene. Now that we have successfully completed our analyses of alternative splicing of *PIK3CD* in white versus AA prostate cancer, we have designed and synthesized chemically modified SSOs to manipulate the alternative splicing of this target. One of the challenges of developing SSOs involves identification of the sequences that block the desired splice sites most efficiently. Therefore, we have designed and synthesized additional oligonucleotides consisting of sequences that span various regions of the splice sites we are interested in targeting plus 4 nucleotides upstream and downstream of these sequences to generate SSOs that block the splice sites most efficiently. We are in the process of testing the effects of these SSOs on *PIK3CD* splicing and prostate cancer cell biology.

In parallel to the work being done in the context of this grant, we have established a number of AA and white prostate cancer patient-derived explants by transferring primary human tumors directly from patients into immunodeficient mice. These models provide invaluable tools, being generated from racially diverse patients and preserving histology patterns from their human counterparts. Such patient-derived explant models can be used with the expectation that results can be applied to the clinical setting. The importance of this cannot be understated: our work represents the first significant collection of prostate cancer patient-derived explants and the first establishment of prostate cancer patient-derived explants from AA patients. Please see ***Figure 9*** at the end of this progress report for a representative example. We will assess the therapeutic efficacy of our aforementioned SSOs in these AA and white prostate cancer patient-derived explant models.

**What opportunities for training and professional development has the project provided?**

Training and professional development has been provided for Jennifer A. Freedman, PhD, Co-investigator and Bonnie LaCroix, Research Analyst I. Dr. Freedman has expanded her expertise in performing all aspects of translational research by writing the GENCADE IRB protocol, designing the GENCADE REDCaP database, creating the GENCADE IRB-approved patient handout and collaborating with members of the Genitourinary Oncology clinical research team to implement the GENCADE Study. In addition, this project has given her an opportunity to collaborate with molecular epidemiologists at our institution to identify variants of stemness-related genes that are predicted to regulate RNA splicing that associate with racial disparities in susceptibility to prostate cancer as well as SNPs of stemness pathway genes that are predicted to regulate RNA splicing, microRNA and transcription that associate with prostate cancer survival. Moreover, Dr. Freedman has gained additional expertise in the design of SSOs and assessment of their effects on splicing and prostate cancer cell biology. Furthermore, she continues to increase her knowledge regarding development of AA and white prostate cancer models and prostate cancer health disparities among racial groups. Finally, this project has provided an opportunity for Dr. Freedman to further develop her skills in scientific management and mentoring of members of the Genitourinary Oncology Laboratory, specifically the Research Analyst I working on this project. Dr. Freedman and Ms. LaCroix attended and presented this study at a poster session at the AACR Cancer Health Disparities Conference last month. Mrs. LaCroix has expanded her technical molecular biology expertise in assessing the effects of SSOs on splicing and prostate cancer cell biology and her knowledge of prostate cancer health disparities among racial groups. Our Genitourinary Oncology Laboratory also has a postdoctoral associate who is working on parallel, but related projects and has benefited from exposure to the research being conducted in the context of this grant. April Deveaux is an African American scientist who conducted her PhD thesis research on genetic/biological aspects of prostate cancer disparities at Howard University Medical Center in Washington DC. She recently completed Medical School at the University of North Carolina, Chapel Hill, and has elected to return to laboratory research for the next phase of her training.

**How were the results disseminated to communities of interest?**

Outreach activities have been undertaken to increase participation, including minority participation, in our GENCADE Study. In collaboration with the Duke Cancer Institute's Office of Health Equity and Disparities (OHED), we have approached patients participating in our annual community Men's Health Fair and have designed, produced and implemented use of our GENCADE IRB-approved patient handout. We have also

worked with OHED to design and implement a training program called JUST ASK, for all staff participants in GENCADE and other clinical trial, centered around culturally sensitive and competent communications with patients about clinical trials.

**What do you plan to do during the next reporting period to accomplish the goals?**

During the next reporting period, we expect to complete our collection of individual patient AA and white prostate cancer tissue specimens and patient-matched blood specimens with accompanying annotation and DNA and RNA isolation. Once complete, we will be performing ancestral genotyping using DNA isolated from the blood specimens and we will be interrogating gene expression, alternative splicing and variation in *cis*-acting splicing elements using DNA and RNA from the tissue specimens. In addition, we expect to complete our assessment of the AR-V7 SSO. Moreover, we expect to complete our assessment of the PIK3CD SSOs we have designed and synthesized. Furthermore, we will begin designing, synthesizing and assessing efficacy of SSOs targeting alternative splicing of *MET*. Such assessments will involve investigating resulting alterations in downstream target expression, transactivation activity, proliferation (for AR studies, androgen-dependent and –independent), migration, invasion, colony formation, apoptosis and sensitivity to inhibitors (for AR studies, enzalutamide). Finally, we will use the AA and white prostate cancer patient-derived explants that we have generated in work that has gone on in parallel with that that has gone on in the context of this grant to assess the therapeutic efficacy of our aforementioned SSOs.

**IMPACT:**

**What was the impact on the development of the principal discipline(s) of the project?**

Collection of individual patient AA and white prostate cancer tissue specimens and patient-matched blood specimens will contribute to the goal of accumulating racially diverse prostate tumor models to assess the biological significance of the factors contributing to the clinical aggressiveness of AA prostate cancer. In addition, development of SSOs to correct aberrant splicing leading to production of AR-V7 and to modulate alternative splicing of *PIK3CD* and *MET* will further our understanding of the contribution of these molecular mechanisms to AA prostate cancer and have the potential to yield novel therapeutic modalities to combat prostate cancer in AA men as well as men of all races with aggressive disease driven by these mechanisms. Finally, in parallel to the work being done in the context of this grant, we have established a number of AA and white prostate cancer patient-derived explants by transferring primary human tumors directly from patients into immunodeficient mice. These models provide invaluable tools, being generated from racially diverse patients and preserving histology patterns from their human counterparts. Such patient-derived explant models can be used with the expectation that results can be applied to the clinical setting. Our work represents the first significant collection of prostate cancer patient-derived explants and the first establishment of prostate cancer patient-derived explants from AA patients. Once these PDXs are fully characterized will be able to make them available to other investigators.

**What was the impact on other disciplines?**

Similar correlations between specific splice variants and aggressiveness have been identified in a wide range of cancers. Therefore, the SSO technology being developed here has the potential to have broader applicability.

**What was the impact on technology transfer?**

Along with our qualified collaborator, a US Patent Application has been filed regarding alternative splicing variants of genes associated with prostate cancer risk and survival (US 2014/0364483 A1). In addition, the variants of stemness-related genes that are predicted to regulate RNA splicing that associate with racial disparities in susceptibility to prostate cancer as well as the SNPs of stemness pathway genes that are predicted to regulate RNA splicing, microRNA and transcription that associate with prostate cancer survival are the subject of a pending patent application at Duke University Medical Center entitled “Biomarkers for the identification of prostate cancer and methods of use”. Furthermore, the SSOs targeting oncogenic androgen receptor and epidermal growth factor receptor RNA isoforms are the subject of a pending patent application at Duke University Medical Center entitled “Splice-switching oligonucleotides and methods of use”. This provisional application is currently being converted for filing of a PCT application.

**What was the impact on society beyond science and technology?**

The outreach activities we have undertaken to increase participation, including minority participation, in our GENCADE Study and our physician-patient informed decision-making and informed consent process in our GENCADE Study will improve public knowledge regarding prostate cancer, prostate cancer health disparities among racial groups and clinical research. In collaboration with DCI OHED we have helped devise a curriculum to train clinicians and other staff participants in clinical trials on culturally sensitive and competent communications with minority patients regarding clinical trials.

**CHANGES/PROBLEMS:**

**Changes in approach and reasons for change.**

In order to increase collection of individual patient AA and white prostate cancer tissue specimens, we have taken advantage of a new strategy to procure tissue from prostatectomies for research that has recently been implemented at our institution. Our institution recently hired a new Department of Pathology chair, Dr. Jiaoti Huang. He had developed a new whole mount strategy to procure tissue from prostatectomies for research at his prior institution and he implemented this strategy at our institution the beginning of May of this year. Since implementation of this strategy, all of the specimens we have obtained have been of sufficient size and tumor content, which has dramatically accelerated our rate of collection and which will continue as we proceed with collection of our remaining specimens.

**Actual or anticipated problems or delays and actions or plans to resolve them.**

We have encountered an unanticipated slower rate of accrual in collecting individual patient AA and white prostate cancer tissue specimens and patient-matched blood specimens. This has been the result of a combination of the record, extremely high volume of patients our Urology clinic has been seeing of late creating unexpected workflow challenges, the challenge of obtaining tissue of sufficient size and tumor content from prostatectomies and delays in obtaining IRB approval of our GENCADE Study at our qualified collaborator's site. We have made changes to address each of the aforementioned hurdles and have successfully implemented each of these changes. First, we have recognized the need to optimize our approach to more seamlessly integrate into the pressured clinical operations at our institution, allowing us to navigate within this environment while not interfering with the need to maximize patient flow. Regarding personnel, we have obtained the support of the Chief of the Division of Urologic Surgery, initiated regular meetings with the urologic surgeons and engaged the nursing staff to incorporate notification of the GENCADE Study into the real-time clinic workflow. In addition, we have designed, produced and implemented use of an IRB-approved GENCADE patient handout, which is given to patients in the context of physician-patient informed decision-making and we have obtained IRB approval to follow up with patients receiving the handout via telephone to obtain informed consent. Second, we have taken advantage of a new strategy to procure tissue from prostatectomies for research that has recently been implemented at our institution. Our institution recently hired a new Department of Pathology chair, Dr. Jiaoti Huang. He had developed a new whole mount strategy to procure tissue from prostatectomies for research at his prior institution and he implemented this strategy at our institution the beginning of May of this year. Since implementation of this strategy, all of the specimens we have obtained have been of sufficient size and tumor content. Third, we successfully obtained IRB approval to collect specimens at our qualified collaborator's site, The George Washington University. Since implementing all of the aforementioned changes, we have already seen an increase in our rate of accrual and anticipate being able to complete collection and analyses of individual patient AA and white prostate cancer tissue specimens and patient-matched blood specimens during the next reporting period.

**Changes that had a significant impact on expenditures.**

Nothing to report.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.**

Nothing to report.

**PRODUCTS:**

**Publications, conference papers, and presentations.**

Peer-reviewed publications:

1. Single nucleotide polymorphisms of stemness pathway genes predicted to regulate RNA splicing, microRNA and transcription are associated with prostate cancer survival, Jennifer A. Freedman[*], Yanru Wang[*], Xuechan Li, Hongliang Liu, Patricia G. Moorman, Daniel J. George, Norman H. Lee, Terry Hyslop, Qingyi Wei[**] and Steven R. Patierno[**], [*]contributed equally as first authors, [**]contributed equally as co-principal investigators, submitted to Clinical Cancer Research
2. Variants of stemness-related genes predicted to regulate RNA splicing associated with racial disparities in susceptibility to prostate cancer, Yanru Wang[*], Jennifer A. Freedman[*], Hongliang Liu, Patricia G. Moorman, Terry Hyslop, Daniel J. George, Norman H. Lee, Steven R. Patierno[**] and Qingyi Wei[**], [*]contributed equally as first authors, [**]contributed equally as co-principal investigators, submitted to Carcinogenesis

Conference presentations:

1. Freedman, J. A.[*], Y. Wang[*], H. Liu, P. G. Moorman, T. Hyslop, D. J. George, N. H. Lee, Q. Wei[*] and S. R. Patierno[*], [*] contributed equally. Single-nucleotide polymorphisms of race-related alternatively spliced genes associate with prostate cancer risk, aggressiveness and/or survival. AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved. Fort Lauderdale, Florida. 2016.
2. Wang, Y.[*], J. A. Freedman[*], H. Liu, P. G. Moorman, T. Hyslop, D. J. George, N. H. Lee, S. R. Patierno[*] and Q. Wei[*], [*] contributed equally. Genetic variants of *CD44*-related stemness pathway genes in risk of prostate cancer. AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved. Fort Lauderdale, Florida. 2016.
3. Freedman, J. A., T. J. Robinson, B. LaCroix, B. M. Patierno, D. J. George, B. A. Sullenger and S. R. Patierno. Development of novel therapeutic splice-switching oligonucleotides against aggressive prostate cancer in men of African descent. Poster Presentation. AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved. Atlanta, Georgia. 2015.
4. Freedman, J. A., T. J. Robinson, B. LaCroix, B. M. Patierno, D. J. George, B. A. Sullenger and S. R. Patierno. Development of novel therapeutic splice-switching oligonucleotides targeting oncogenic RNA isoforms driving aggressive prostate cancer. Poster Presentation. AACR-JCA Joint Conference on Breakthroughs in Cancer Research: From Biology to Therapeutics. Maui, Hawaii. 2015.
5. Freedman, J. A., T. J. Robinson, B. LaCroix, B. M. Patierno, D. J. George, B. A. Sullenger and S. R. Patierno. Development of novel therapeutic splice-switching oligonucleotides against aggressive prostate cancer in men of African descent. Poster Presentation. DCI Scientific Retreat. Durham, NC. 2015.

**Website(s) or other Internet site(s).**

Nothing to report.

**Technologies or techniques.**

SSOs to correct aberrant splicing leading to production of AR-V7, to manipulate *PIK3CD* and *MET* alternative splicing and to drive production of inhibitory androgen receptor and epidermal growth factor receptor isoforms have been developed and, once complete, we plan to submit this research data for publication making our scientific discovery open to the scientific community. In addition, in parallel to the work being done in the context of this grant, we have established a number of AA and white prostate cancer patient-derived explants by transferring primary human tumors directly from patients into immunodeficient mice. These models provide invaluable tools, being generated from racially diverse patients and preserving histology patterns from their human counterparts. Such patient-derived explant models can be used with the expectation that results can be applied to the clinical setting. Our work represents the first significant collection of prostate cancer patient-derived explants and the first establishment of prostate cancer patient-derived explants from AA patients. We plan to submit this research data for publication making our scientific discovery open to the scientific community.

**Inventions, patent applications, and/or licenses.**

Along with our qualified collaborator, a US Patent Application has been filed regarding alternative splicing variants of genes associated with prostate cancer risk and survival (US 2014/0364483 A1). In addition, the

variants of stemness-related genes that are predicted to regulate RNA splicing that associate with racial disparities in susceptibility to prostate cancer as well as the SNPs of stemness pathway genes that are predicted to regulate RNA splicing, microRNA and transcription that associate with prostate cancer survival are the subject of a pending patent application at Duke University Medical Center entitled “Biomarkers for the identification of prostate cancer and methods of use”. Furthermore, the SSOs targeting oncogenic androgen receptor and epidermal growth factor receptor RNA isoforms are the subject of a pending patent application at Duke University Medical Center entitled “Splice-switching oligonucleotides and methods of use”. This provisional application is currently being converted for filing of a PCT application.

**Other products.**

Nothing to report.

## PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS:

**What individuals have worked on the project?**

| Name: | **Steven Patierno, PhD, PI** |
|---|---|
| Project Role: | Principal Investigator |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | No change |
| Contribution to Project: | Provided senior level oversight and direction, contributed to implementation of the GENCADE study |
| Funding Support | P30CA014236-42 See attached other support/ DOD |
| | |

| Name: | **Daniel J George, MD** |
|---|---|
| Project Role: | Co-Investigator |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | No change |
| Contribution to Project: | Contributed to implementation of the GENCADE study, contributed to oversight and direction |
| Funding Support | See attached other support/ DOD |
| | |

| Name: | **Susan K Murphy,PhD** |
|---|---|
| Project Role: | Co-Investigator |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | No change |
| Contribution to Project: | Planned pilot experiments to generate epigenetic data using prostate cancer cell lines derived from African American and white patients and GENCADE specimens collected to date |
| Funding Support | See attached other support/ DOD |
| | |

| Name: | **Jennifer A Freedman, PhD** |
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| | |
|---|---|
| Project Role: | Co-Investigator |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 1.1 |
| Contribution to Project: | Wrote GENCADE IRB protocol, designed GENCADE REDCaP database, created GENCADE IRB-approved patient handout and contributed to implementation of the GENCADE study in collaboration with the Genitourinary Oncology clinical research team, designed splice-switching oligonucleotides to manipulate PIK3CD alternative splicing, managed and mentored Research Analyst I, Bonnie LaCroix |
| Funding Support | See attached other support/ DOD |
| | |

| | |
|---|---|
| Name: | **Jason A Somarelli** |
| Project Role: | Postdoctoral Associate |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | No change |
| Contribution to Project: | No contribution this reporting period |
| Funding Support | Prostate Cancer Foundation Award |

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|---|---|
| Name: | **Yuan Wu** |
| Project Role: | Biostatitician |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | No change |
| Contribution to Project: | No contribution this reporting period |
| Funding Support | |
| | |

| | |
|---|---|
| Name: | **Zhiqing Huang** |
| Project Role: | Senior Research Scientitist |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | No change |
| Contribution to Project: | No contribution this reporting period |
| Funding Support | |
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|---|---|
| Name: | **Carole Grenier** |
| Project Role: | Research Analyst I |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | No change |
| Contribution to Project: | No contribution this reporting period |
| Funding Support | |
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|---|---|
| Name: | **Norman Lee, PhD** |
| Project Role: | GWU PI |
| Researcher Identifier (e.g. ORCID ID): | NHLEE1 |
| Nearest person month worked: | 1.2 |
| Contribution to Project: | Coordinated efforts between Duke and GWU. Was responsible for experimental design of primer pairs and oversaw all laboratory activities |
| Funding Support | See attached other support/ DOD |
| | |

| | |
|---|---|
| Name: | **Bi-Dar Wang, PhD** |
| Project Role: | GWU Co-Investigator |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | No change |
| Contribution to Project: | Performed RTPCR testing and validation of primer pairs for amplification of short and long isoforms of PI3KCD in AA and EA PCa cell lines and patient specimens |
| Funding Support | See attached other support/ DOD |
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|---|---|
| Name: | **Ramez Andrawls, MD** |
| Project Role: | GWU Co-Investigator |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | No change |
| Contribution to Project: | Provided prostate cancer specimens for testing of primer pairs |
| Funding Support | See attached other support/ DOD |
| | |

**Collaborating Organization – George Washington University Medical Center**

**RT-PCR validation of L and S variants of PIK3CD in PCa cell lines and patient specimens.** The Lee lab has validated a series of primer pairs for the amplification of the short (S) and long (L) isoforms of PIK3CD in a panel of AA (MDA PCa 2b, E006AA) and EA PCa cell lines (PC-3, LNCaP and VCaP) as well as validation in prostate cancer specimens of AA or EA origin. Primer pairs for exons 20 through 24 consistently amplify 547bp (L form) and 379bp products (S form missing exon 23); and primer pairs for exons 20 through 25 consistently amplify 685bp (L form) and 517bp products (S). S isoform is consistently more highly expressed in AA cell lines, and L isoform is consistently more highly expressed in EA lines. Similar results are seen in patient specimens. Having validated these primer pairs, the next step will be to use these primer pairs to assess the ability of splice switching oligos (SSO), being developed by our Duke collaborators, to switch isoform usage in PCa cell lines and to test the functional consequences of isoform switching. Lastly, patient consent form in accordance to Duke protocols/procedures is being reviewed and finalized by GWU IRB.

**RT-PCR validation of the long and short splice variants of PIK3CD is in AA and EA PCa cell lines.** To further evaluate the functional role(s) of the splice variants of PIK3CD *in vitro*, we first applied RT-PCR assays

to investigate whether the long and short splice variants of PIK3CD were expressed in PCa cell line models. Four commercially available PCa cell lines, including PC-3, LNCaP, VCaP and MDA PCa 2b (ATCC, Manassas, VA) were used in this RT-PCR validation experiment. PC-3, VCaP were derived from EA PCa patients with bone metastasis, and LNCaP was derived from EA patients with lymph node metastasis, while MDA PCa 2b was derived from AA patient with bone metastasis. RNA samples were purified from the EA and AA PCa cell lines using Qiagen RNeasy mini kit (Qiagen, Valencia, CA) and were subjected to the RT-PCR assays. RT-PCR results showed that the short variant of PIK3CD was enriched in AA PCa cell line MDA PCa 2b, whereas the EA cell lines (PC-3, LNCaP and VCaP) exhibited higher expression of PIK3CD long variant (**Figure 1a**). To further quantify the expression level of long and short variants of PIK3CD in the EA and AA PCa cell lines, EA cell line VCaP and AA cell line MDA PCa 2b (both derived from bone-metastasis) were selected to compare their expression levels of PIK3CD-L and –S variants. Forward primer on exon 20 and reverse primers on exon 24 and 25 were designed for the RT-PCR validation of the long and short splice variants of PIK3CD. Using the primer pair targeting exon 20 and 25, the long variant (685bp) and short variant (517bp, exon 23 is missing) were amplified by RT-PCR reactions. Quantification analysis showed that the ratios of PIK3CD-S/PIK3CD-L were 0.33 and 9.01 in VCaP and MDA PCa 2b, respectively (**Figure 1b, left panel**). By using another primer pairs for amplifying exon 20-24 transcripts, our RT-PCR results again showed a higher PIK3CD-S (379bp)/PIK3CD-L (547bp) ratio in MDA PCa 2b (ratio of 3.02) compared to VCaP (ratio of 0.80) (**Figure 1b, right panel**). These results were consistent with our RT-PCR results in AA and EA PCa clinical samples, confirming that higher level of PIK3CD short variant is expressed in AA PCa compared to EA PCa.

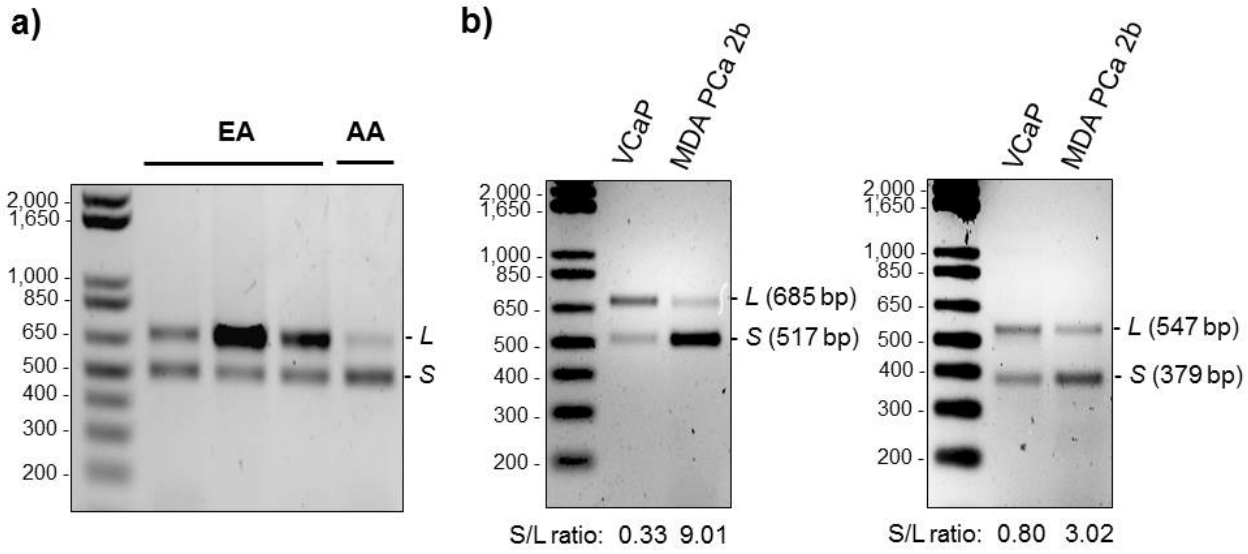



**Figure 1.** RT-PCR validation of PIK3CD splice variants in EA and AA PCa cell lines. **(a)** RNA samples purified from commercially available EA PCa cell lines (PC-3, LNCaP and VCaP; lane 1-3) and AA PCa cell line (MDA PCa 2b, lane 4) were used in the RT-PCR assays. EA cell lines expressed both long and short variants (long variant as major isoform) and AA cell line has higher expression of PIK3CD short variant. The primers targeting exon 20 and 24 were used in the PCR reactions. **(b)** Quantification analysis of long and short variants of PIK3CD in EA cell line VCaP and AA cell line MDA PCa 2b. Primers for exon 20 and 25 were used to amplify the long (685bp) and short (517bp) variants of PIK3CD (left panel). Primers for exon 20 and 24 were used to amplify the long (547bp) and short (379bp) variants of PIK3CD (left panel). The S/L ratio was determined by measuring the gel images of PIK3CD-S and –L transcripts using Image J program.

**References:**

1. Gibas Z, Becher R, Kawinski E, Horoszewicz J, Sandberg AA. A high-resolution study of chromosome changes in a human prostatic carcinoma cell line (LNCaP). Cancer Genet Cytogenet 1984;11: 399-404.

2. Navone NM, Olive M, Ozen M*, et al.* Establishment of two human prostate cancer cell lines derived from a single bone metastasis. Clin Cancer Res 1997;3: 2493-500.

**ABSTRACT (approximately 200 words):**

These studies address the urgent need to elucidate the molecular mechanisms underlying the more aggressive prostate cancer biology in African American (AA) men. To address this urgent need, we have opened our GENomics of Cancer DispartiEs Study to collect AA and white prostate cancer blood and tissue specimens of varying Gleason grade, DNA/RNA from each and annotated data for further study. In addition, we have identified variants of stemness-related genes that are predicted to regulate RNA splicing that associate with racial disparities in susceptibility to prostate cancer and variants of stemness pathway genes that are predicted to regulate RNA splicing, microRNA and transcription that associate with prostate cancer survival. Furthermore, we have developed a splice-switching oligonucleotide that inhibits production of an oncogenic androgen receptor isoform at the RNA and protein level. Corresponding with this biochemical effect, this oligonucleotide inhibits colony forming ability and proliferation of prostate cancer cells. Ultimately, this study will establish the genetic/epigenetic/post-transcriptional differences between AA and white prostate cancer and their relevance to tumor biology, which will pave the way toward novel biomarkers and/or molecular targets that will reduce prostate cancer health disparities for AAs and improve outcomes for men of all races with aggressive disease.

***Figure 1.*** Research flowchart to identify (A) top SNPs in African descendants, (B) top SNPs in non-Hispanic whites and heterogeneity between the two racial populations.

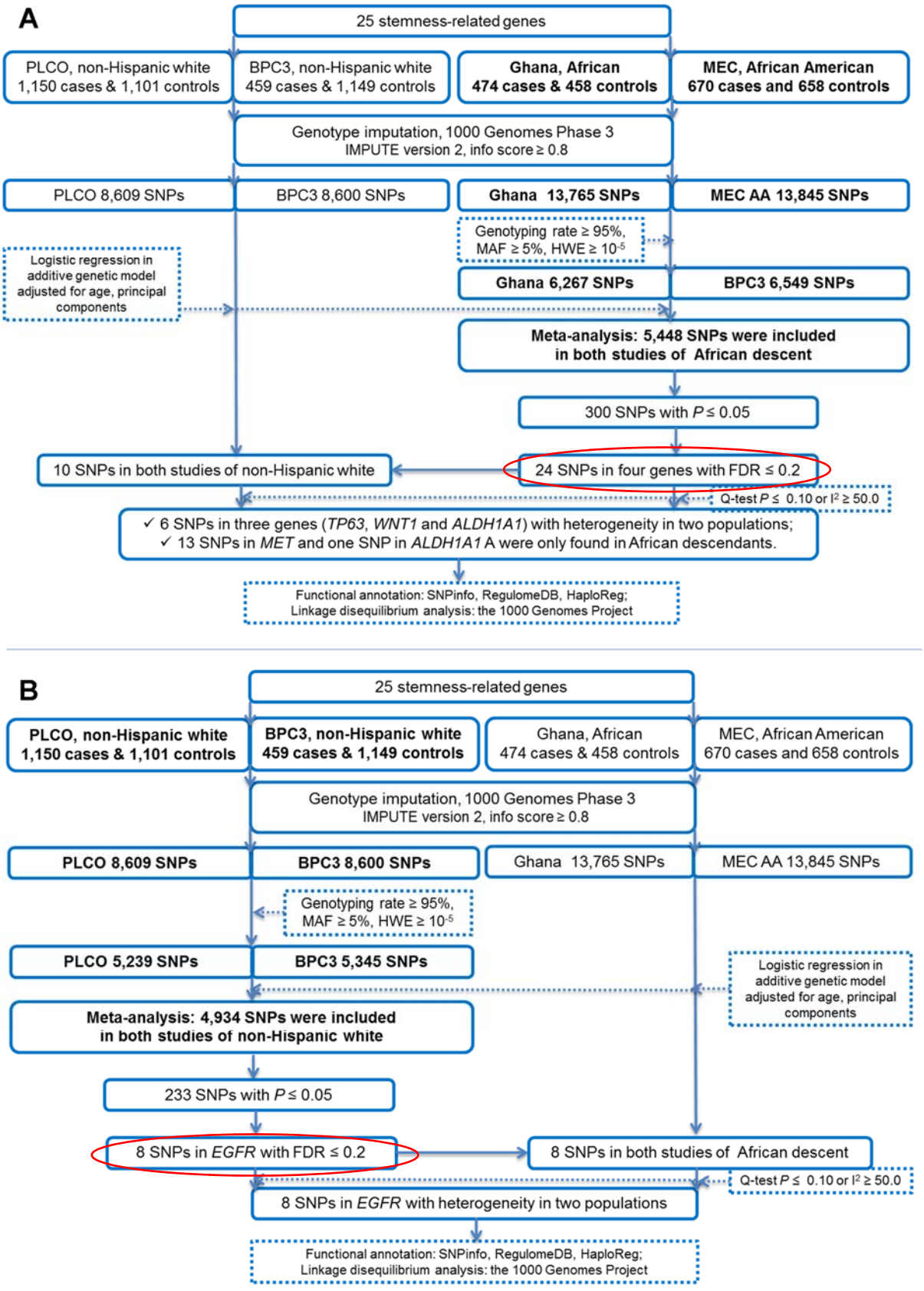

A
25 stemness-related genes
PLCO, non-Hispanic white
1,150 cases & 1,101 controls
BPC3, non-Hispanic white
459 cases & 1,149 controls
Ghana, African
474 cases & 458 controls
MEC, African American
670 cases and 658 controls
Genotype imputation, 1000 Genomes Phase 3
IMPUTE version 2, info score ≥ 0.8
PLCO 8,609 SNPs
BPC3 8,600 SNPs
Ghana 13,765 SNPs
MEC AA 13,845 SNPs
Genotyping rate ≥ 95%,
MAF ≥ 5%, HWE ≥ 10-5
Logistic regression in
additive genetic model
adjusted for age, principal
components
Ghana 6,267 SNPs
BPC3 6,549 SNPs
Meta-analysis: 5,448 SNPs were included
in both studies of African descent
300 SNPs with P ≤ 0.05
10 SNPs in both studies of non-Hispanic white
24 SNPs in four genes with FDR ≤ 0.2
Q-test P ≤ 0.10 or I2 ≥ 50.0
✓ 6 SNPs in three genes (TP63, WNT1 and ALDH1A1) with heterogeneity in two populations;
✓ 13 SNPs in MET and one SNP in ALDH1A1 A were only found in African descendants.
Functional annotation: SNPinfo, RegulomeDB, HaploReg;
Linkage disequilibrium analysis: the 1000 Genomes Project
B
25 stemness-related genes
PLCO, non-Hispanic white
1,150 cases & 1,101 controls
BPC3, non-Hispanic white
459 cases & 1,149 controls
Ghana, African
474 cases & 458 controls
MEC, African American
670 cases and 658 controls
Genotype imputation, 1000 Genomes Phase 3
IMPUTE version 2, info score ≥ 0.8
PLCO 8,609 SNPs
BPC3 8,600 SNPs
Ghana 13,765 SNPs
MEC AA 13,845 SNPs
Genotyping rate ≥ 95%,
MAF ≥ 5%, HWE ≥ 10-5
PLCO 5,239 SNPs
BPC3 5,345 SNPs
Logistic regression in
additive genetic model
adjusted for age, principal
components
Meta-analysis: 4,934 SNPs were included
in both studies of non-Hispanic white
233 SNPs with P ≤ 0.05
8 SNPs in EGFR with FDR ≤ 0.2
8 SNPs in both studies of African descent
Q-test P ≤ 0.10 or I2 ≥ 50.0
8 SNPs in EGFR with heterogeneity in two populations
Functional annotation: SNPinfo, RegulomeDB, HaploReg;
Linkage disequilibrium analysis: the 1000 Genomes Project

***Figure 2.*** Manhattan plots of the four studies and the meta-analysis results of the two racial populations. The red horizontal line indicates $P = 0.05$ and the blue line indicates FDR = 0.2. (A) 6,549 common SNPs from the Ghana study. (B) 6,267 common SNPs from the MEC AA study. (C) The meta-analysis of 5,448 SNPs in two studies of African descendants. (D) 5,239 common SNPs from the PLCO study. (E) 5,345 common SNPs from the BPC3 study. (F) The meta-analysis of 4,934 SNPs in two studies of non-Hispanic whites.

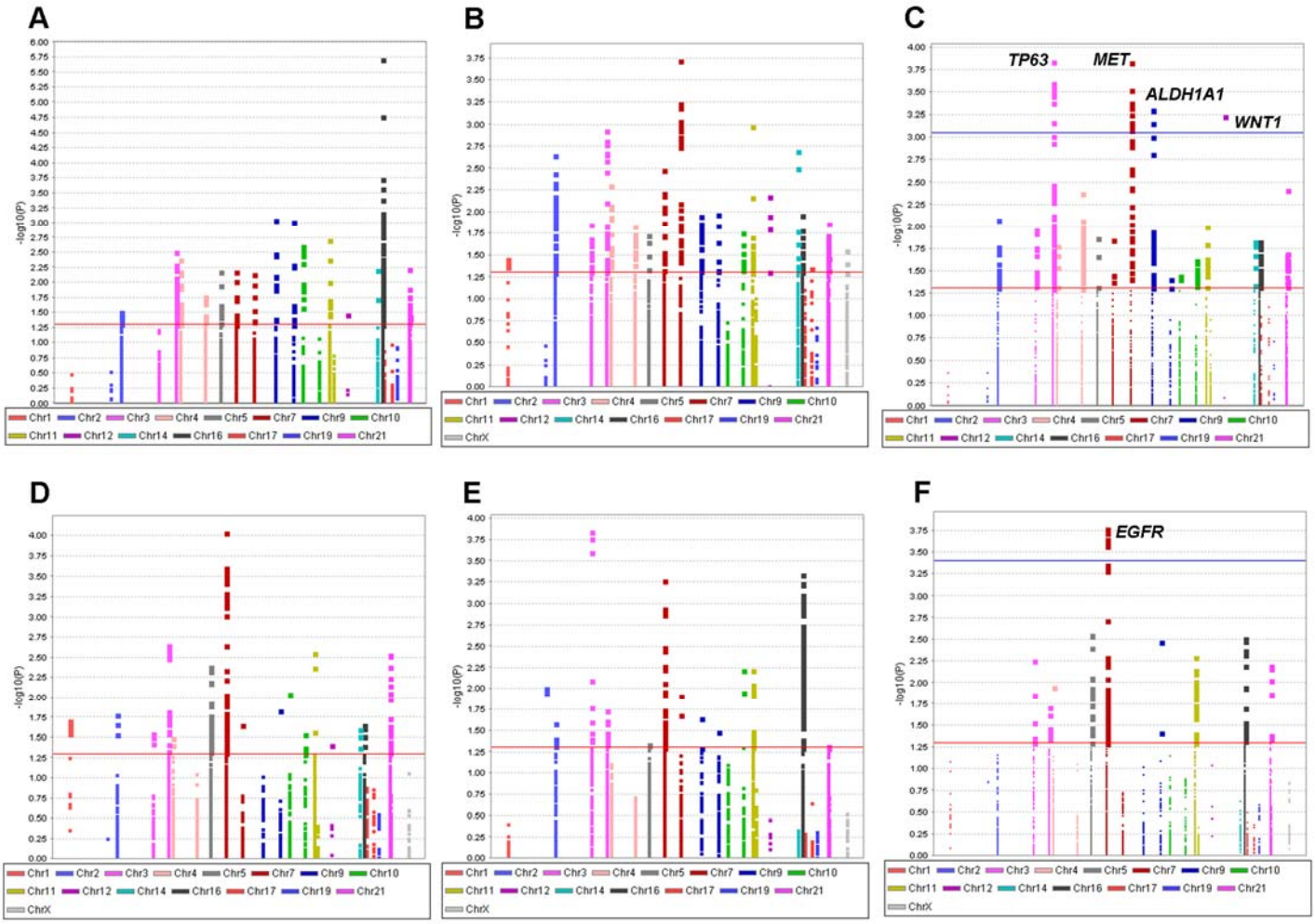


***Figure 3.*** Overview of the top SNPs in the two racial populations and linkage disequilibrium (LD) analysis based on the 1000 Genomes Project Phase 3 database. Genome browser of gene regions from UCSC browser (NCBI37/hg19) (A) *TP63*, (C) *MET*, (E) *ALDH1A1* and (G) *EGFR*. LD analysis in Africans: (B) 8 SNPs in *TP63*, (D) 13 SNPs in *MET* and (F) 2 SNPs in *ALDH1A1*. LD analysis in Europeans: (H) 8 SNPs in *EGFR*.

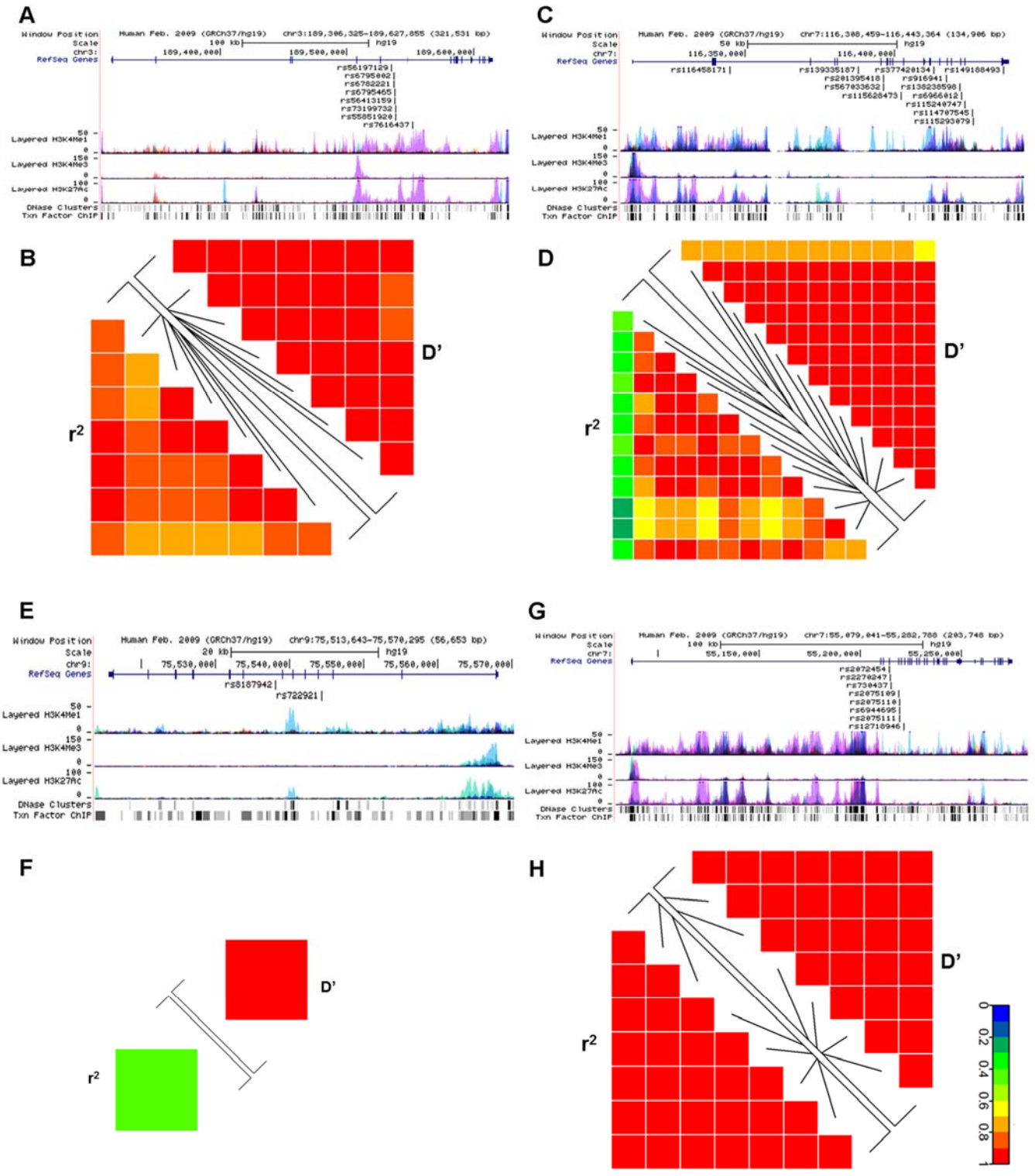


***Figure 4.*** Correlation between SNPs and their corresponding mRNA expression level in lymphoblastoid cell lines from the HapMap 3 Project. (A) rs116458171 and *MET* mRNA expression level in 326 Africans, and (B) rs2072454 and *EGFR* mRNA expression level in 107 Europeans.

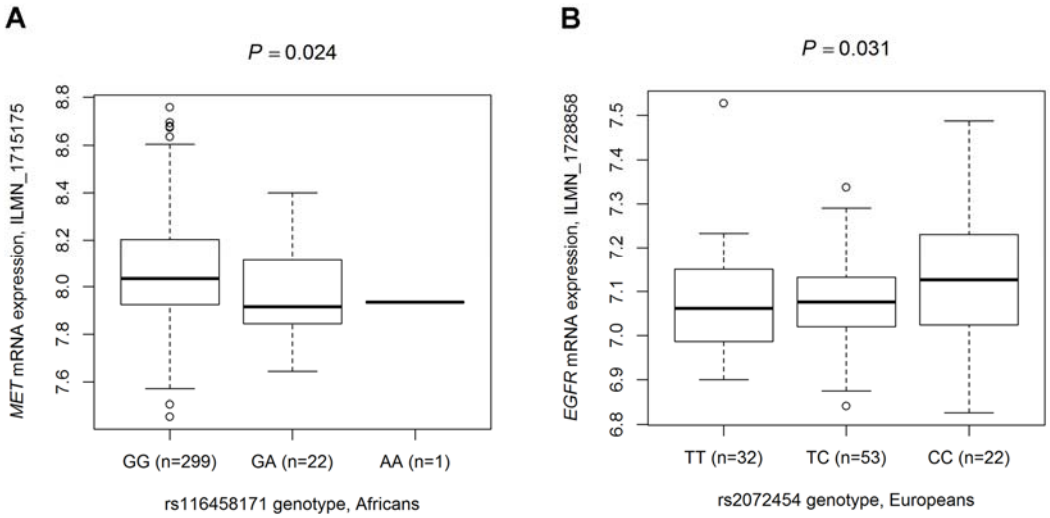


***Table 1.*** The top SNPs associated with prostate cancer risk in study populations (after correction by FDR ≤ 0.2 in each population).

| SNP | CHR | BP | Gene | Allele [a] | EAF[b] | OR (95% CI)[c] | *P*[c] | FDR [d] | eQTL in African [e] | eQTL in European[e] |
|---|---|---|---|---|---|---|---|---|---|---|
| **24 Top SNPs in African Descendants** | | | | | | | | | | |
| rs56197129 | 3 | 189534843 | *TP63* | C/A | 0.17 | 1.35 (1.15-1.60) | 3.16E-04 | 0.170 | 0.801 | 0.766 |
| rs6795002 | 3 | 189537082 | *TP63* | T/A | 0.18 | 1.35 (1.15-1.59) | 2.51E-04 | 0.170 | 0.798 | 0.682 |
| rs6782221 | 3 | 189537156 | *TP63* | G/A | 0.16 | 1.35 (1.14-1.60) | 4.17E-04 | 0.170 | 0.887 | 0.766 |
| rs6795465 | 3 | 189537521 | *TP63* | T/C | 0.16 | 1.36 (1.15-1.61) | 3.46E-04 | 0.170 | 0.981 | 0.959 |
| rs56413159 | 3 | 189537609 | *TP63* | G/C | 0.16 | 1.34 (1.13-1.59) | 6.72E-04 | 0.170 | 0.995 | 0.766 |
| rs73199732 | 3 | 189538240 | *TP63* | C/G | 0.17 | 1.36 (1.15-1.60) | 2.88E-04 | 0.170 | 0.883 | 0.766 |
| rs55851920 | 3 | 189538560 | *TP63* | T/C | 0.17 | 1.36 (1.15-1.60) | 2.77E-04 | 0.170 | 0.883 | 0.671 |
| rs7616437 | 3 | 189551420 | *TP63* | A/G | 0.18 | 1.37 (1.16-1.60) | 1.44E-04 | 0.170 | 0.597 | 0.912 |
| rs116458171* | 7 | 116345048 | *MET* | G/A | 0.04 | 1.65 (1.23-2.21) | 7.18E-04 | 0.170 | 0.024 | -- |
| rs139335187* | 7 | 116387806 | *MET* | ACTCGGC/TCTGG/- | 0.05 | 1.66 (1.26-2.19) | 2.96E-04 | 0.170 | 0.339 | -- |
| rs201395418* | 7 | 116396348 | *MET* | TAA/- | 0.05 | 1.58 (1.21-2.05) | 6.66E-04 | 0.170 | 0.445 | -- |
| rs567033632* | 7 | 116396353 | *MET* | A/C | 0.05 | 1.58 (1.21-2.05) | 6.66E-04 | 0.170 | 0.445 | -- |
| rs115628473* | 7 | 116402123 | *MET* | A/C | 0.05 | 1.63 (1.24-2.14) | 4.11E-04 | 0.170 | 0.259 | -- |
| rs377420134* | 7 | 116413125 | *MET* | A/- | 0.05 | 1.56 (1.21-2.02) | 7.10E-04 | 0.170 | 0.231 | -- |
| rs916941* | 7 | 116417210 | *MET* | T/C | 0.05 | 1.57 (1.20-2.04) | 8.10E-04 | 0.184 | 0.445 | -- |
| rs138238598* | 7 | 116421879 | *MET* | G/A | 0.05 | 1.65 (1.26-2.17) | 2.94E-04 | 0.170 | 0.384 | -- |
| rs6966012* | 7 | 116422214 | *MET* | C/T | 0.05 | 1.59 (1.22-2.07) | 5.54E-04 | 0.170 | 0.502 | -- |
| rs115240747* | 7 | 116423320 | *MET* | A/G | 0.05 | 1.59 (1.23-2.06) | 4.57E-04 | 0.170 | 0.231 | -- |
| rs114707545* | 7 | 116425961 | *MET* | C/A | 0.06 | 1.62 (1.26-2.08) | 1.47E-04 | 0.170 | 0.410 | -- |
| rs115293079* | 7 | 116426224 | *MET* | C/T | 0.06 | 1.62 (1.26-2.08) | 1.47E-04 | 0.170 | 0.410 | -- |
| rs149188493* | 7 | 116436394 | *MET* | C/A | 0.05 | 1.60 (1.23-2.09) | 4.70E-04 | 0.170 | 0.597 | -- |
| rs8187942* | 9 | 75538072 | *ALDH1A1* | C/G | 0.06 | 0.60 (0.44-0.80) | 4.90E-04 | 0.170 | 0.294 | -- |
| rs722921 | 9 | 75544299 | *ALDH1A1* | T/A | 0.18 | 0.73 (0.61-0.88) | 6.84E-04 | 0.170 | 0.848 | 0.679 |
| rs855723 | 12 | 49370547 | *WNT1* | A/G | 0.48 | 1.26 (1.10-1.43) | 5.80E-04 | 0.170 | 0.863 | 0.038 |
| **8 Top SNPs in Non-Hispanic Whites** | | | | | | | | | | |
| rs2072454 | 7 | 55214348 | *EGFR* | T/C | 0.48 | 1.23 (1.10-1.37) | 2.66E-04 | 0.164 | 0.361 | 0.031 |
| rs2270247 | 7 | 55214647 | *EGFR* | T/G | 0.48 | 1.23 (1.10-1.37) | 2.47E-04 | 0.164 | 0.367 | 0.031 |
| rs730437 | 7 | 55215018 | *EGFR* | C/A | 0.48 | 1.23 (1.10-1.37) | 2.47E-04 | 0.164 | 0.367 | 0.031 |
| rs2075109 | 7 | 55218903 | *EGFR* | C/T | 0.48 | 1.23 (1.11-1.37) | 1.74E-04 | 0.164 | 0.675 | 0.031 |
| rs2075110 | 7 | 55219159 | *EGFR* | T/C | 0.48 | 1.23 (1.11-1.38) | 1.69E-04 | 0.164 | 0.675 | 0.031 |
| rs6944695 | 7 | 55219290 | *EGFR* | C/T | 0.48 | 1.23 (1.10-1.37) | 2.21E-04 | 0.164 | 0.910 | 0.031 |
| rs2075111 | 7 | 55219307 | *EGFR* | G/C | 0.48 | 1.23 (1.10-1.37) | 2.21E-04 | 0.164 | 0.313 | 0.031 |
| rs12718946 | 7 | 55221447 | *EGFR* | G/C | 0.48 | 1.23 (1.10-1.37) | 1.86E-04 | 0.164 | 0.675 | 0.031 |

Abbreviations: CHR: chromosome; EAF: effect allele frequency; OR: odd ratio; CI: confidence interval; FDR: false discovery rate; eQTL: expression quantitative trait loci;

* 13 SNPs in *MET* and one SNP in *ALDH1A1* were found only in populations of African descent.

[a] Referring to "common allele/effect allele";

[b] EAF in controls;

[c] Meta-analysis of the two studies in the same population; Logistic regression analysis was adjusted for age and principal components in each study;

[d] FDR was calculated in each population.

[e] eQTL were analyzed based on datasets from the HapMap3 project with 107 Europeans or 326 Africans.

***Table 2.*** Functional annotation of the top SNPs from the study populations of two races.

| SNP | Gene | Location | SNP info[a] | Regulome DB score[b] | HaploReg v4.1[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Promoter histone marks | Enhancer histone marks | DNase | Protein bound | Motif changed | eQTL hits |
| **24 Top SNPs in African Descendants** | | | | | | | | | | |
| rs56197129 | *TP63* | Intron | -- | -- | -- | 4 tissues | -- | -- | Evi-1 | -- |
| rs6795002* | *TP63* | Intron | -- | 6 | -- | IPSC, SKIN, LNG | -- | -- | 16 altered motifs | -- |
| rs6782221 | *TP63* | Intron | -- | 6 | -- | IPSC, SKIN, LNG | -- | -- | Pou2f2,Pou 3f3,XBP-1 | -- |
| rs6795465 | *TP63* | Intron | -- | 6 | -- | SKIN, LNG | -- | -- | 4 altered motifs | -- |
| rs56413159 | *TP63* | Intron | -- | -- | -- | SKIN, LNG | LNG | -- | 7 altered motifs | -- |
| rs73199732 | *TP63* | Intron | -- | 4 | LNG | 6 tissues | LNG | -- | Pax-4,RFX5 | -- |
| rs55851920 | *TP63* | Intron | -- | -- | BLD | 4 tissues | Pou5f1, Sox | | | -- |
| rs7616437 | *TP63* | Intron | -- | -- | SKIN | BRST, SKIN | SKIN | -- | -- | 1 hit |
| rs116458171* | *MET* | Intron | -- | 5 | -- | 7 tissues | SKIN,MUS | -- | 4 altered motifs | -- |
| rs139335187 | *MET* | Intron | -- | -- | -- | -- | -- | -- | GCNF,RXRA | -- |
| rs201395418 | *MET* | Intron | -- | 6 | -- | -- | -- | -- | 12 altered motifs | -- |
| rs567033632 | *MET* | Intron | -- | 6 | -- | -- | -- | -- | -- | -- |
| rs115628473 | *MET* | Intron | -- | 5 | -- | BRN, LNG | KID | -- | LBP-1 | -- |
| rs377420134 | *MET* | Intron | -- | 5 | -- | -- | -- | -- | -- | -- |
| rs916941* | *MET* | Intron | -- | 2b | LNG | 11 tissues | 12 tissues | CFOS | 10 altered motifs | -- |
| rs138238598 | *MET* | Intron | -- | -- | -- | 13 tissues | SKIN,GI | -- | BDP1,TBX5,Zfx | -- |
| rs6966012 | *MET* | Intron | -- | 2b | ESC, IPSC | 14 tissues | 12 tissues | -- | 4 altered motifs | -- |
| rs115240747 | *MET* | Intron | -- | -- | -- | 11 tissues | -- | -- | Sox,ZEB1 | -- |
| rs114707545 | *MET* | Intron | -- | 5 | -- | STRM, SKIN, BONE | -- | -- | -- | -- |
| rs115293079 | *MET* | Intron | -- | 3a | -- | STRM, SKIN, BONE | LNG,CRVX | CTCF | DEC,GR,SRF | -- |
| rs149188493 | *MET* | 3'-UTR | -- | 5 | -- | 4 tissues | PLCNT | -- | 4 altered motifs | -- |
| rs8187942* | *ALDH1A1* | Intron | -- | 5 | -- | -- | LNG | POL2 | GR | -- |
| rs722921* | *ALDH1A1* | Intron | -- | 6 | -- | SKIN, GI, KID | -- | -- | 9 altered motifs | -- |
| rs855723* | *WNT1* | 5'-Upstream | TFBS | 3a | -- | BLD | -- | CTCF | 5 altered motifs | 4 hits |
| **8 Top SNPs in Non-Hispanic Whites** | | | | | | | | | | |
| rs2072454* | *EGFR* | Synonymous | Splicing | 6 | -- | FAT, SKIN | -- | -- | -- | 1 hit |
| rs2270247 | *EGFR* | Intron | -- | -- | -- | ESDR, FAT, SKIN | PLCNT | -- | LBP-1,Rad21 | 1 hit |
| rs730437 | *EGFR* | Intron | -- | 5 | -- | STRM, SKIN | -- | -- | 14 altered motifs | 1 hit |
| rs2075109 | *EGFR* | Intron | -- | 5 | -- | FAT, MUS, VAS | MUS,MUS | -- | FXR,Sox | 2 hits |
| rs2075110 | *EGFR* | Intron | -- | 5 | -- | FAT, MUS, VAS | MUS | -- | 9 altered motifs | 2 hits |
| rs6944695 | *EGFR* | Intron | -- | 6 | -- | MUS, VAS | -- | -- | 22 altered motifs | -- |
| rs2075111 | *EGFR* | Intron | -- | -- | -- | MUS, VAS | -- | -- | PPAR | -- |
| rs12718946 | *EGFR* | Intron | -- | 5 | -- | MUS | -- | RAD21 | 7 altered motifs | -- |

*Selected SNPs based on LD analysis; [a]http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm; [b]http://www.regulomedb.org/index; [c]http://www.broadinstitute.org/mammals/haploreg/haploreg.php.

[*]Notes: In the *MET* gene, three SNPs (rs139335187, rs201395418 and rs567033632) were in high LD ($r^2$ > 0.8) with rs13223756, and four SNPs (rs377420134, rs115240747, rs114707545 and rs115293079) were in moderate LD ($r^2$ =0.63-0.72) with rs13223756 based on 1000 Genomes Phase 3 African population. The SNP rs13223756 was located in the exonic region in *MET* and predicted to be involved in splicing regulation.

In *ALDH1A1*, rs722921 and rs13959 were in moderate LD ($r^2$=0.69) based on 1000 Genomes Phase 3 African population. The SNP rs13959 was located in the exonic region in *ALDH1A1* and predicted to be involved in splicing regulation.

***Table 3.*** The SNPs showed heterogeneity between the study populations of two races.

| SNP | CHR | Position | Gene | Location | MEC and Ghana, African descent | | | | PLCO and BPC3, non-Hispanic white | | | | Heterogeneity [c] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Allele [a] | EAF | OR (95% CI)[b] | *P*[c] | Allele [a] | EAF | OR (95% CI)[b] | *P*[c] | Q | $I^2$ |
| **6 Top SNPs in African Descendants with Heterogeneity** | | | | | | | | | | | | | | |
| rs56197129 | 3 | 189534842 | *TP63* | Intron | C/A | 0.17 | 1.35 (1.15-1.60) | 3.16E-04 | C/A | 0.12 | 1.14 (0.97-1.34) | 0.110 | 0.148 | 52.3 |
| rs6795002 | 3 | 189537081 | *TP63* | Intron | T/A | 0.18 | 1.35 (1.15-1.59) | 2.51E-04 | T/A | 0.13 | 1.14 (0.97-1.34) | 0.114 | 0.142 | 53.6 |
| rs55851920 | 3 | 189538559 | *TP63* | Intron | T/C | 0.17 | 1.36 (1.15-1.60) | 2.77E-04 | T/C | 0.12 | 1.15 (0.98-1.35) | 0.097 | 0.152 | 51.2 |
| rs7616437 | 3 | 189551419 | *TP63* | Intron | A/G | 0.18 | 1.37 (1.16-1.60) | 1.44E-04 | A/G | 0.19 | 1.09 (0.87-1.36) | 0.461 | 0.106 | 61.7 |
| rs722921 | 9 | 75544298 | *ALDH1A1* | Intron | T/A | 0.18 | 0.73 (0.61-0.88) | 0.001 | A/T | 0.46 | 1.00 (0.83-1.21) | 0.991 | 0.019 | 81.7 |
| rs855723 | 12 | 49370546 | *WNT1* | 5'-Upstream | A/G | 0.48 | 1.26 (1.10-1.43) | 0.001 | A/G | 0.15 | 1.04 (0.90-1.22) | 0.589 | 0.067 | 70.1 |
| **8 Top SNPs in Non-Hispanic Whites with Heterogeneity** | | | | | | | | | | | | | | |
| rs2072454 | 7 | 55214348 | *EGFR* | Exon-Synonymous | T/C | 0.51 | 0.89 (0.72-1.10) | 0.285 | T/C | 0.48 | 1.23 (1.10-1.37) | 2.66E-04 | 0.009 | 85.6 |
| rs2270247 | 7 | 55214647 | *EGFR* | Intron | T/G | 0.50 | 0.89 (0.73-1.10) | 0.293 | T/G | 0.48 | 1.23 (1.10-1.37) | 2.47E-04 | 0.008 | 85.6 |
| rs730437 | 7 | 55215018 | *EGFR* | Intron | C/A | 0.51 | 0.89 (0.73-1.10) | 0.291 | C/A | 0.48 | 1.23 (1.10-1.37) | 2.47E-04 | 0.008 | 85.7 |
| rs2075109 | 7 | 55218903 | *EGFR* | Intron | C/T | 0.45 | 0.98 (0.86-1.11) | 0.755 | C/T | 0.48 | 1.23 (1.11-1.37) | 1.74E-04 | 0.007 | 86.0 |
| rs2075110 | 7 | 55219159 | *EGFR* | Intron | T/C | 0.37 | 0.96 (0.84-1.09) | 0.506 | T/C | 0.48 | 1.23 (1.11-1.38) | 1.69E-04 | 0.004 | 88.3 |
| rs6944695 | 7 | 55219290 | *EGFR* | Intron | C/T | 0.50 | 0.93 (0.82-1.06) | 0.281 | C/T | 0.48 | 1.23 (1.10-1.37) | 2.21E-04 | 0.001 | 90.4 |
| rs2075111 | 7 | 55219307 | *EGFR* | Intron | G/C | 0.50 | 0.93 (0.82-1.06) | 0.264 | G/C | 0.48 | 1.23 (1.10-1.37) | 2.21E-04 | 0.001 | 90.6 |
| rs12718946 | 7 | 55221447 | *EGFR* | Intron | G/C | 0.42 | 0.91 (0.80-1.04) | 0.160 | G/C | 0.48 | 1.23 (1.10-1.37) | 1.86E-04 | 0.001 | 91.8 |

Abbreviations: CHR: chromosome; EAF: effect allele frequency; OR: odd ratio; CI: confidence interval;
[a] Referring to "common allele/effect allele";
[b] Meta-analysis of the two studies in each population; Logistic regression analysis was adjusted for age and principal components in each studies;
[c] Heterogeneity were defined as Q-test $P \leq 0.100$ or $I^2 \geq 50.0\%$.

***Figure 5***. Kaplan-Meier survival curves of prostate cancer patients according to combined risk alleles of the five independent and significant SNPs.

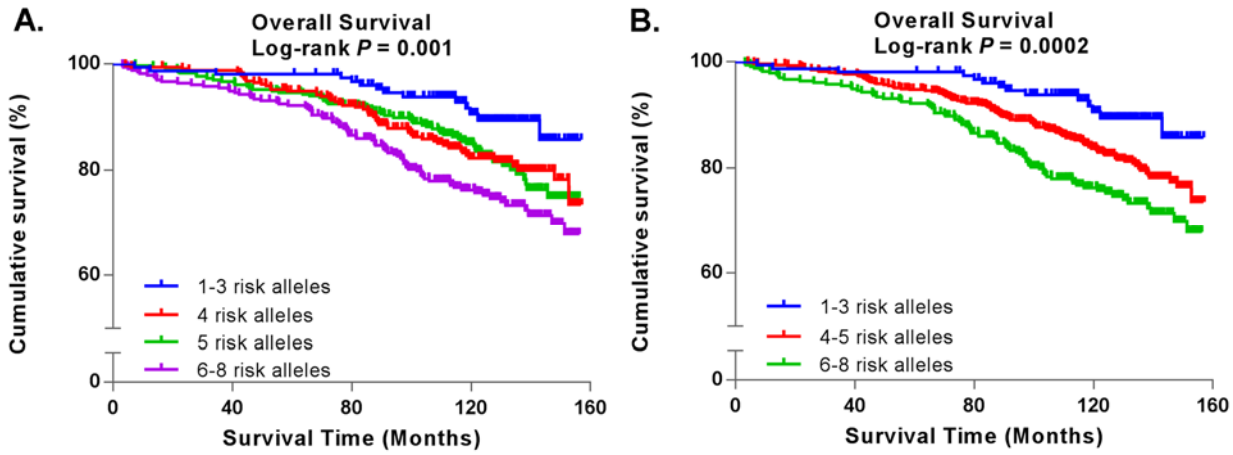


***Figure 6.*** Receiver-operating characteristic (ROC) curves for prediction of prostate cancer survival based on only clinical characteristics (age, Gleason score and tumor stage) or additionally combined effect genotypes, (A) time-dependent area under curve (AUC) and (B) ROC curves corresponding to the time point of ten-year.

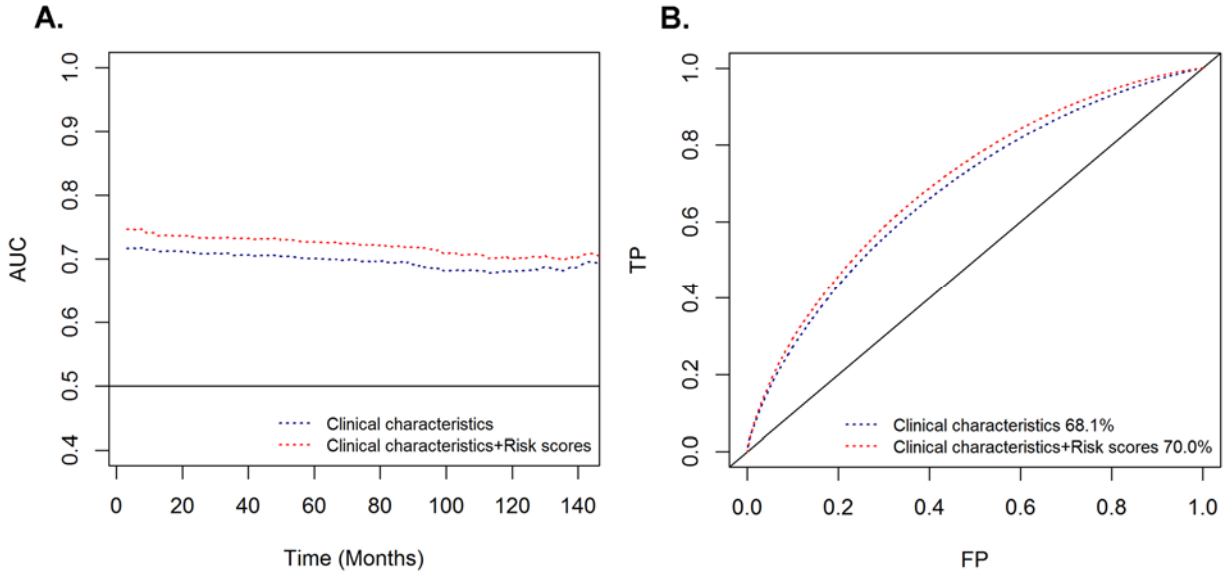


***Figure 7.*** Correlation between the five independent and significant SNPs and the relative mRNA expression in lymphoblastoid cell lines of 716 individuals from HapMap 3 Project including 107 CEU, 242 CHB, 41 MEX and 326 AFR populations. (A) rs35605 and *ABCC1* (reporter: ILMN_1802404); (B) rs212091 and *ABCC1* (reporter: ILMN_1802404); (C) rs9666607 and *CD44* (reporter: ILMN_1803429); (D) rs11009151 and *ITGB1* (reporter: ILMN_1723467); (E) rs1058587 and *GDF15* (reporter: ILMN_1763658).

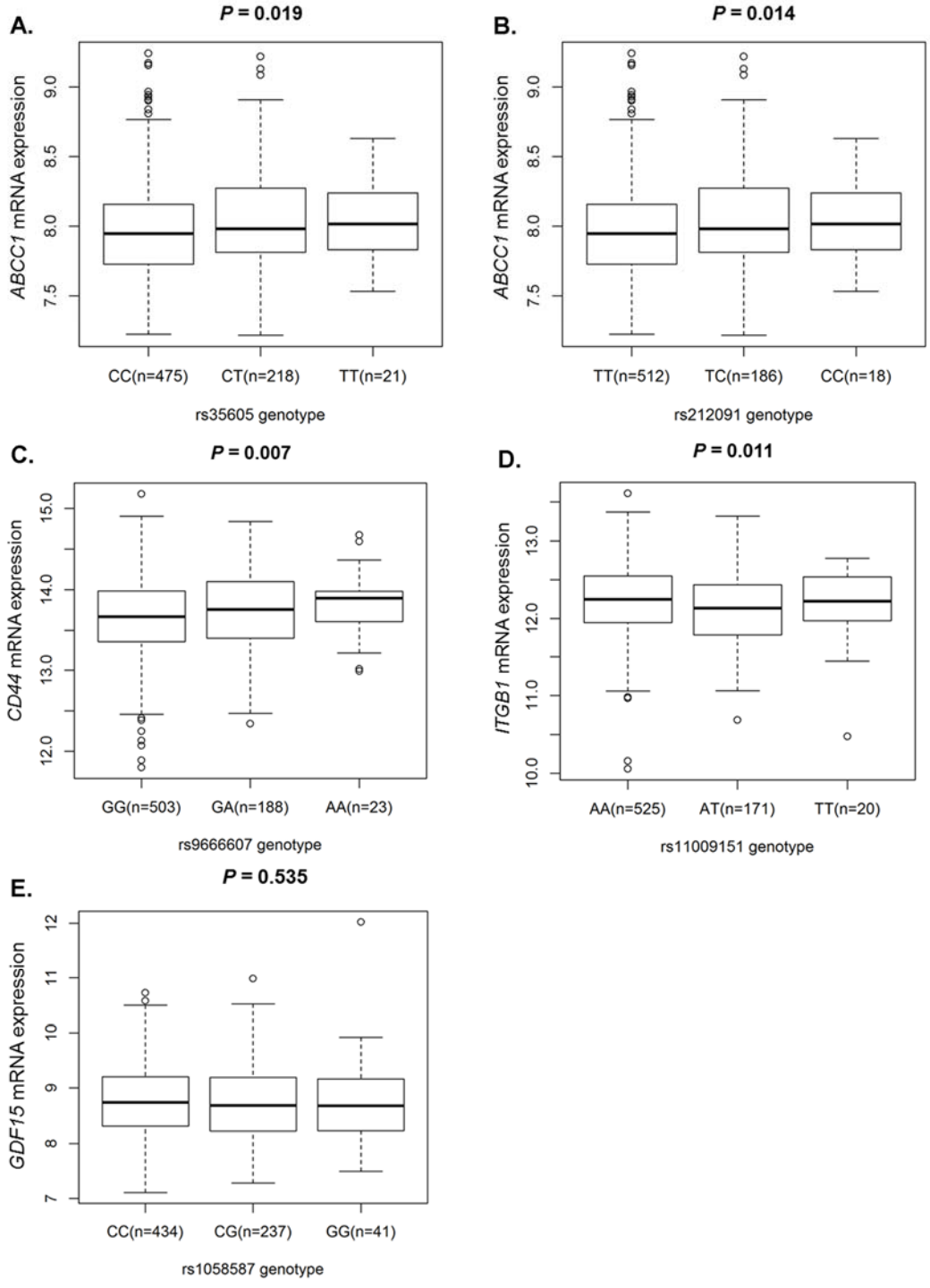


***Table 4.*** Clinical characteristics of the prostate cancer PLCO study in overall survival.

| Characteristics | Frequency | | Univariate Analysis | | Multivariate Analysis [a] | |
|---|---|---|---|---|---|---|
| | All | Deaths (%) | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Overall | 1150 | 215 (18.7) | | | | |
| Age (years) | | | | | | |
| Median (range) | 67 (55-81) | | | | | |
| < 67 | 544 | 70 (12.9) | 1.00 | | 1.00 | |
| ≥ 67 | 606 | 145 (23.9) | 2.24 (1.68-2.98) | < 0.001 | 1.80 (1.34-2.42) | < 0.001 |
| PSA before diagnosis (ng/ml) | | | | | | |
| Median (range) | 6.1 (0.05-1137) | | | | | |
| < 6.1 | 572 | 89 (15.6) | 1.00 | | 1.00 | |
| ≥ 6.1 | 578 | 126 (21.8) | 1.38 (1.05-1.81) | 0.021 | 0.98 (0.74-1.30) | 0.887 |
| Gleason score | | | | | | |
| 2≤&≤ 6 | 567 | 96 (16.9) | 1.00 | | 1.00 | |
| = 7 | 464 | 76 (16.4) | 1.19 (0.88-1.61) | 0.254 | 1.23 (0.90-1.70) | 0.199 |
| ≥8 | 114 | 41 (36.0) | 3.13 (2.17-4.53) | < 0.001 | 2.52 (1.68-3.77) | < 0.001 |
| *Missing* | 5 | | | | | |
| Stage | | | | | | |
| I/II | 913 | 153 (16.7) | 1.00 | | 1.00 | |
| III/IV | 237 | 62 (26.2) | 1.62 (1.20-2.17) | 0.002 | 1.76 (1.26-2.46) | 0.001 |
| Aggressiveness [b] | | | | | | |
| Non-aggressive | 489 | 78 (16.0) | 1.00 | | 1.00 | |
| Aggressive | 659 | 137 (20.8) | 1.65 (1.24-2.18) | 0.001 | 1.89 (1.40-2.56) | < 0.001 |
| *Missing* | 2 | | | | | |
| Types of treatments | | | | | | |
| Radical prostatectomy | 614 | 75 (12.2) | 1.00 | | 1.00 | |
| Radiotherapy alone | 194 | 46 (23.7) | 2.00 (1.39-2.89) | < 0.001 | 2.06 (1.39-3.06) | < 0.001 |
| Radiotherapy + Endocrine therapy | 202 | 40 (19.8) | 1.96 (1.34-2.88) | 0.001 | 1.56 (1.03-2.36) | 0.035 |
| Endocrine therapy alone | 54 | 29 (53.7) | 7.21 (4.68-11.09) | < 0.001 | 4.28 (2.64-6.92) | < 0.001 |
| Other treatments | 86 | 25 (29.1) | 2.53 (1.61-3.98) | < 0.001 | 2.68 (1.65-4.36) | < 0.001 |

Abbreviations: PLCO: The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; HR: Hazards ratio; CI: Confidence interval; PSA: Prostate specific antigen.

[a] Multivariate cox regression analyses were adjusted for age, Gleason score, PSA level, stage and primary treatments. In subgroup of "Aggressiveness", we included age, PSA level, and primary treatment for adjustments;

[b] Non-aggressive: cases with a Gleason score <7 and stage < III; Aggressive: cases with a Gleason score ≥ 7 or stage ≥ III.

***Table 5.*** The five independent and significant SNPs with potential functions.

| SNP | Gene | Chr. | Position | Location | Allele [a] | EAF | SNPinfo [b] | RegulomeDB [c] | HR (95% CI) [d] | *P* [d] | FPRP [d] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| rs11009151 | *ITGB1* | 10 | 33219109 | Intron | A/T | 0.17 | -- | 1f | 1.31 (1.03-1.67) | 0.026 | 0.188 |
| rs9666607 | *CD44* | 11 | 35226155 | Exon | G/A | 0.31 | Splicing | 5 | 1.28 (1.04-1.58) | 0.018 | 0.137 |
| rs35605 | *ABCC1* | 16 | 16162019 | Exon | C/T | 0.16 | Splicing | 1f | 0.71 (0.53-0.94) | 0.018 | 0.138 |
| rs212091 | *ABCC1* | 16 | 16236650 | 3'UTR | T/C | 0.15 | miRNA | 7 | 0.61 (0.45-0.83) | 0.002 | 0.016 |
| rs1058587 | *GDF15* | 19 | 18499422 | Exon | C/G | 0.26 | nsSNP | 4 | 1.29 (1.05-1.59) | 0.015 | 0.116 |

Abbreviations: SNP: Single nucleotide polymorphism; nsSNP: Non-synonymous SNP; Chr.: Chromosome; EAF: Effect allele frequency; HR: Hazards ratio; CI: Confidence interval; UTR: Untranslated region; FPRP: False positive report probability;

[a] Reference/effect allele;

[b] SNPinfo, http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm ;

[c] RegulomeDB, http://regulome.stanford.edu/. SNPs with predicted scores of "1" were considered as functional.

[d] Multivariate cox regression analyses were adjusted for age, Gleason score, stage and primary treatments;

***Table 6.*** Combined analysis of the five independent and significant SNPs.

| Number of risk alleles [a] | Frequency | | Multivariate analysis [b] | | AIC [c] |
|---|---|---|---|---|---|
| | All | Deaths (%) | HR (95% CI) | *P* | |
| 1-2 | 37 | 2 (5.4) [d] | 1.00 | | |
| 3 | 115 | 12 (10.4) | 3.39 (0.75-15.38) | 0.114 | |
| 4 | 311 | 55 (17.7) | 6.00 (1.44-24.92) | 0.014 | |
| 5 | 333 | 60 (18.0) | 6.50 (1.56-27.03) | 0.010 | |
| 6 | 213 | 53 (24.9) | 10.58 (2.54-44.09) | 0.001 | |
| 7 | 93 | 22 (23.7) | 8.99 (2.09-38.73) | 0.003 | |
| 8 | 26 | 7 (26.9) | 12.26 (2.50-60.19) | 0.002 | |
| Trend | | | 1.30 (1.18-1.44) | 2.81E-07 | 2655.77 |
| | | | | | |
| 1-3 | 152 | 14 (9.2) | 1.00 | | |
| 4 | 311 | 55 (17.7) | 2.35 (1.30-4.27) | 0.005 | |
| 5 | 333 | 60 (18.0) | 2.54 (1.40-4.61) | 0.002 | |
| 6-8 | 332 | 82 (24.7) | 4.00 (2.25-7.12) | 2.41E-06 | |
| Trend | | | 1.43 (1.25-1.65) | 4.04E-07 | 2654.96 |
| | | | | | |
| 1-3 | 152 | 14 (9.2) | 1.00 | | |
| 4-5 | 644 | 115 (17.9) | 2.45 (1.39-4.30) | 0.002 | |
| 6-8 | 332 | 82 (24.7) | 4.00 (2.25-7.12) | 2.41E-06 | |
| Trend | | | 1.82 (1.46-2.27) | 1.05E-07 | 2652.77 |

Abbreviations: SNP: Single nucleotide polymorphism; HR: Hazards ratio; CI: Confidence interval; AIC, Akaike information criterion.

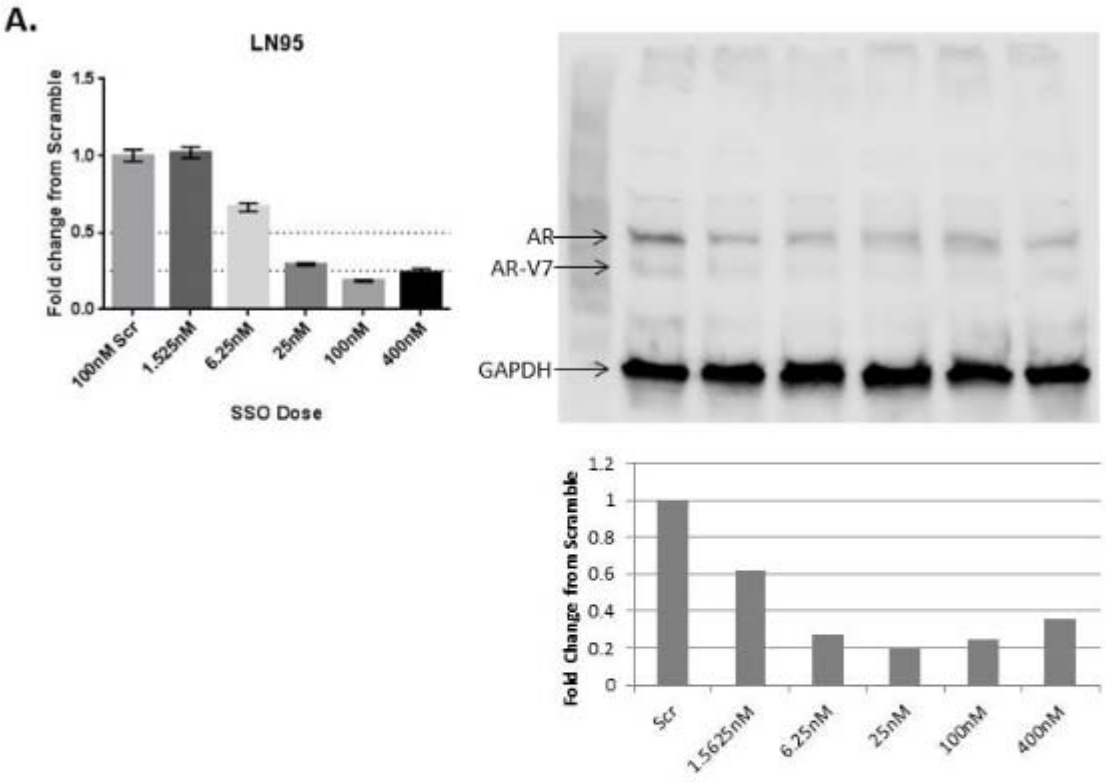
[a] Risk alleles were rs11009151 T, rs9666607 A, rs35605 C, rs212091 T and rs1058587 G;

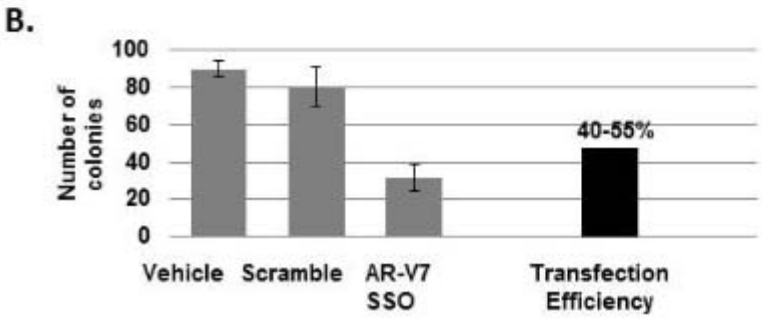
[b] Multivariate cox regression analyses were adjusted for age, Gleason score, stage and primary treatments;

[c] AIC in the trend model of multivariate cox regression analyses;

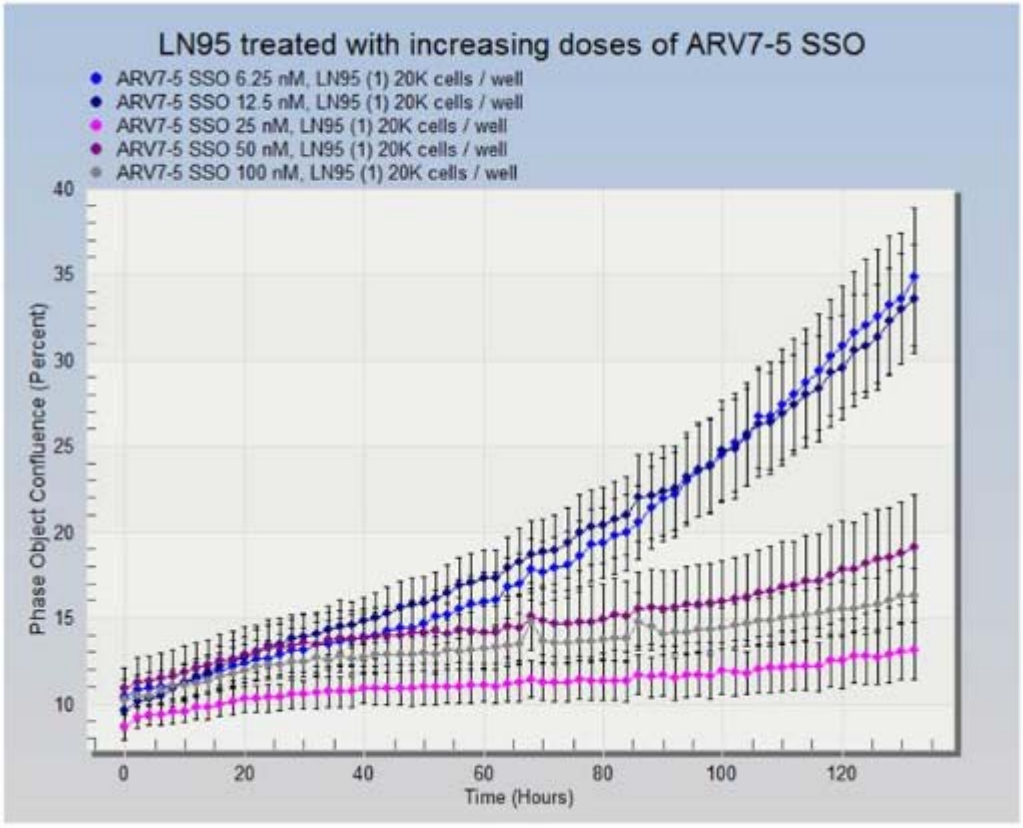
[d] Two patients reached the endpoint were both with two risk alleles.

***Figure 8.*** A. Representative example of AR-V7 SSO inhibiting AR-V7 RNA (left) and protein (right) in a dose-dependent manner in LN95 prostate cancer cells derived from a white prostate cancer patient. B. AR-V7 SSO inhibits colony forming ability in MDA PCa 2b prostate cancer cells derived from an AA prostate cancer patient. C. AR-V7 SSO inhibits proliferation of LN95 prostate cancer cells. D and E. AR-V7 SSO inhibits proliferation of LN95 prostate cancer cells in the presence of enzalutmide. Scr = control scrambled SSO.

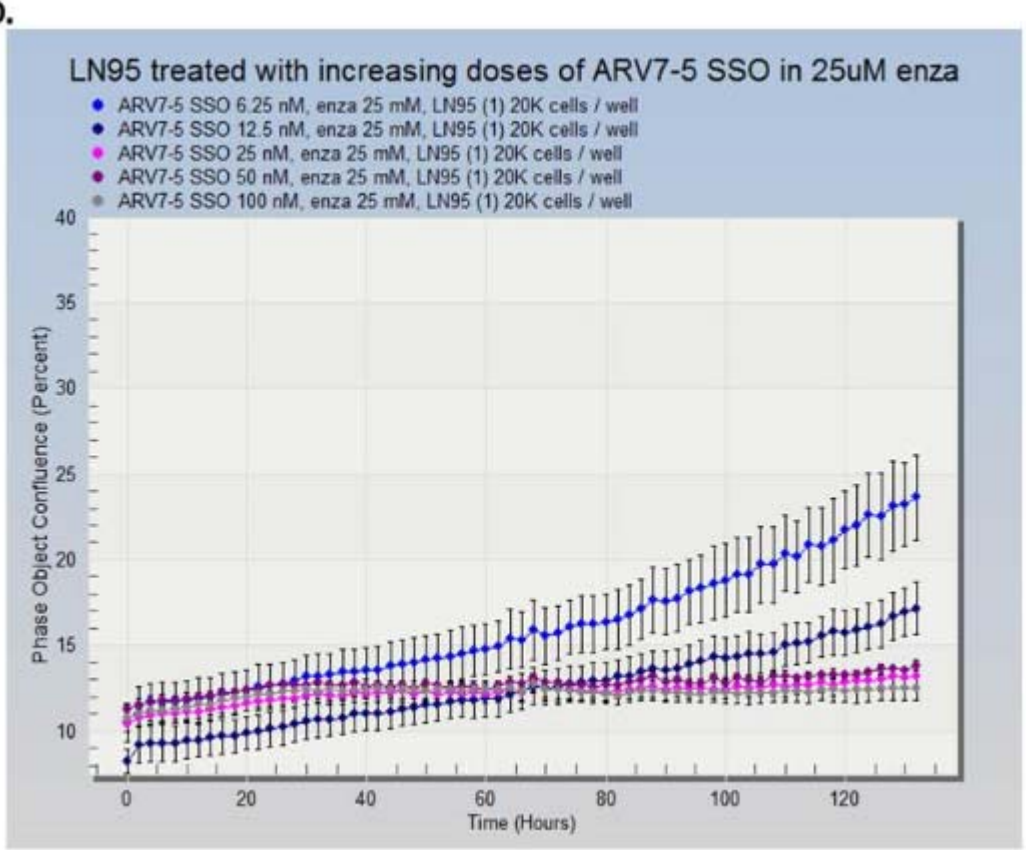


C.


LN95 treated with increasing doses of ARV7-5 SSO
ARV7-5 SSO 6.25 nM, LN95 (1) 20K cells / well
ARV7-5 SSO 12.5 nM, LN95 (1) 20K cells / well
ARV7-5 SSO 25 nM, LN95 (1) 20K cells / well
ARV7-5 SSO 50 nM, LN95 (1) 20K cells / well
ARV7-5 SSO 100 nM, LN95 (1) 20K cells / well
Phase Object Confluence (Percent)
Time (Hours)

D.


LN95 treated with increasing doses of ARV7-5 SSO in 25uM enza
ARV7-5 SSO 6.25 nM, enza 25 mM, LN95 (1) 20K cells / well
ARV7-5 SSO 12.5 nM, enza 25 mM, LN95 (1) 20K cells / well
ARV7-5 SSO 25 nM, enza 25 mM, LN95 (1) 20K cells / well
ARV7-5 SSO 50 nM, enza 25 mM, LN95 (1) 20K cells / well
ARV7-5 SSO 100 nM, enza 25 mM, LN95 (1) 20K cells / well
Phase Object Confluence (Percent)
Time (Hours)

**E.**

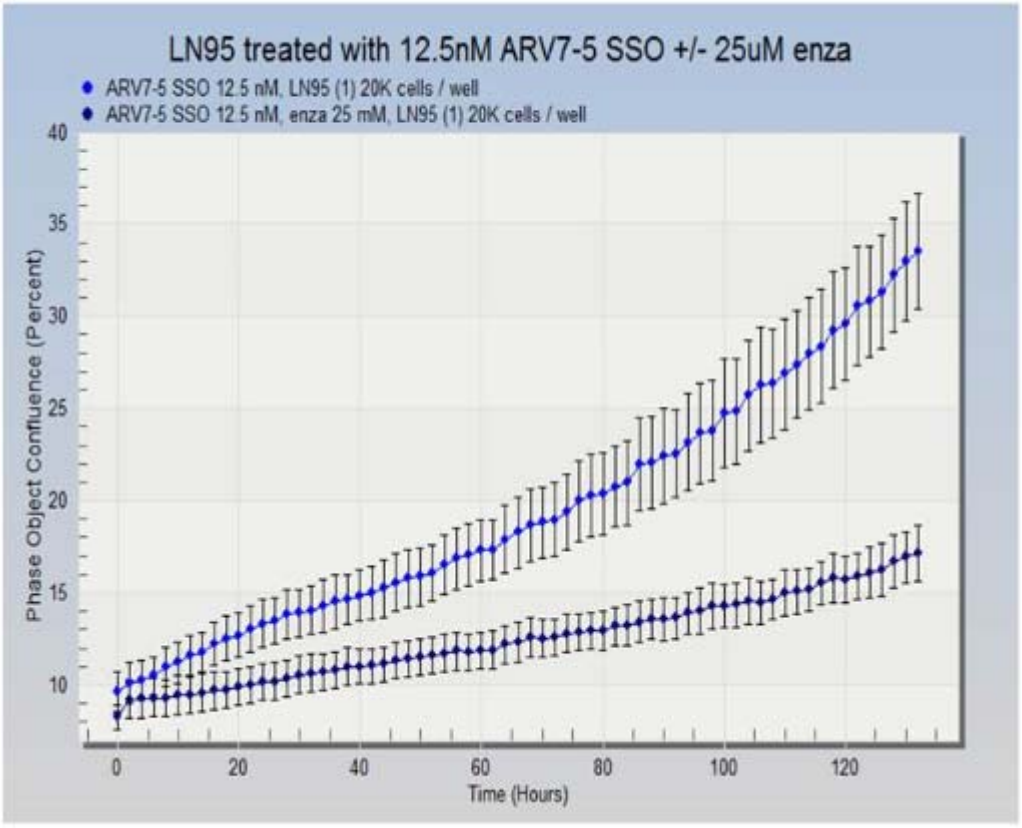

LN95 treated with 12.5nM ARV7-5 SSO +/- 25uM enza
ARV7-5 SSO 12.5 nM, LN95 (1) 20K cells / well
ARV7-5 SSO 12.5 nM, enza 25 mM, LN95 (1) 20K cells / well
Phase Object Confluence (Percent)
40
35
30
25
20
15
10
0
20
40
60
80
100
120
Time (Hours)

***Figure 9.*** A. Representative example of a renal capsule injected prostate cancer patient-derived explant we have generated from a core prostate sample resected from an AA patient diagnosed with adenocarcinoma of the prostate (Gleason 10), metastatic to lymph nodes and penis undergoing a pelvic exenteration. B. H&E sections of the patient tumor (left) and the patient-derived explant (right) showing consistent histopathological features, including very poor differentiation, prominent eosinophilic nucleoli and pale chromatin.